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DATE: Tuesday, February 15, 2005

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| | | <i>DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ</i> | |
| <input type="checkbox"/> | L6 | ompa same (tissue plasminogen activator or tpa or t-pa or k2s or kringle adj2 2 adj2 serine) | 3 |
| | | <i>DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ</i> | |
| <input type="checkbox"/> | L5 | L4 and nucleic acid | 74 |
| <input type="checkbox"/> | L4 | L3 and signal peptide | 80 |
| <input type="checkbox"/> | L3 | ompa and (tissue plasminogen activator or tpa or t-pa or k2s or kringle adj2 2 adj2 serine) | 112 |
| <input type="checkbox"/> | L2 | L1 and signal adj1 peptide | 4 |
| <input type="checkbox"/> | L1 | ompa same (tissue plasminogen activator or tpa or t-pa or k2s or kringle adj2 2 adj2 serine) | 6 |

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: US 6083715 A

Using default format because multiple data bases are involved.

L1: Entry 1 of 6

File: USPT

Jul 4, 2000

US-PAT-NO: 6083715

DOCUMENT-IDENTIFIER: US 6083715 A

**** See image for Certificate of Correction ****

TITLE: Methods for producing heterologous disulfide bond-containing polypeptides in bacterial cells

DATE-ISSUED: July 4, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|------------|-------|----------|---------|
| Georgiou; George | Austin | TX | | |
| Oiu; Ji | Austin | TX | | |
| Bessette; Paul | Austin | TX | | |
| Swartz; James | Menlo Park | CA | | |

US-CL-CURRENT: 435/69.1; 435/252.1, 435/252.8, 435/320.1, 435/69.7, 536/23.1, 536/23.4

| | | | | | | | | | | | | |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. D. |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|

☐ 2. Document ID: US 6027888 A

L1: Entry 2 of 6

File: USPT

Feb 22, 2000

US-PAT-NO: 6027888

DOCUMENT-IDENTIFIER: US 6027888 A

TITLE: Methods for producing soluble, biologically-active disulfide-bond containing eukaryotic proteins in bacterial cells

DATE-ISSUED: February 22, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|---------------|-------|----------|---------|
| Georgiou; George | Austin | TX | | |
| Ostermeier; Marc | State College | PA | | |

US-CL-CURRENT: 435/6; 435/243, 435/320.1, 435/69.1, 435/91.1, 530/350, 536/23.2,
536/23.5

ABSTRACT:

Disclosed are methods of producing eukaryotic disulfide bond-containing polypeptides in bacterial hosts, and compositions resulting therefrom. Co-expression of a eukaryotic foldase and a disulfide bond-containing polypeptide in a bacterial host cell is demonstrated. In particular embodiments, the methods have been used to produce mammalian pancreatic trypsin inhibitor and tissue plasminogen activator (tPA) in soluble, biologically-active forms, which are isolatable from the bacterial periplasm. Also disclosed are expression systems, recombinant vectors, and transformed host cells.

40 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMC | Draw. D |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|-----|---------|
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|-----|---------|

☐ 3. Document ID: US 5789199 A

L1: Entry 3 of 6

File: USPT

Aug 4, 1998

US-PAT-NO: 5789199

DOCUMENT-IDENTIFIER: US 5789199 A

**** See image for Certificate of Correction ****

TITLE: Process for bacterial production of polypeptides

DATE-ISSUED: August 4, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|------------|-------|----------|---------|
| Joly; John C. | San Mateo | CA | | |
| Swartz; James R. | Menlo Park | CA | | |

US-CL-CURRENT: 435/69.1; 435/252.3, 435/252.33

ABSTRACT:

A process is provided for producing a heterologous polypeptide in bacteria. This process comprises, in a first step, culturing bacterial cells that lack their native *pstS* gene and comprise nucleic acid encoding a PstS variant having an amino acid variation within the phosphate-binding region of the corresponding native PstS, nucleic acid encoding a DsbA or DsbC protein, nucleic acid encoding the heterologous polypeptide, a signal sequence for secretion of both the DsbA or DsbC protein and the heterologous polypeptide, an inducible promoter for the nucleic acid encoding the DsbA or DsbC protein, and an alkaline phosphatase promoter for the nucleic acid encoding the heterologous polypeptide. The nucleic acid encoding a PstS variant is under the transcriptional control of the wild-type *pstS* gene promoter. The second step of the process involves recovering the heterologous polypeptide from the periplasm or the culture medium.

32 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMIC | Draw. D. |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|

☐ 4. Document ID: US 5639635 A

L1: Entry 4 of 6

File: USPT

Jun 17, 1997

US-PAT-NO: 5639635
DOCUMENT-IDENTIFIER: US 5639635 A

TITLE: Process for bacterial production of polypeptides

DATE-ISSUED: June 17, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|------------|-------|----------|---------|
| Joly; John C. | San Mateo | CA | | |
| Swartz; James R. | Menlo Park | CA | | |

US-CL-CURRENT: 435/69.1; 536/23.5, 536/23.6, 536/23.7

ABSTRACT:

A process is provided for producing a heterologous polypeptide in bacteria, which process comprises:

(a) culturing bacterial cells, which cells comprise nucleic acid encoding a DsbA or DsbC protein, nucleic acid encoding the heterologous polypeptide, a signal sequence for secretion of both the DsbA or DsbC protein and the heterologous polypeptide, and an inducible promoter for both the nucleic acid encoding the DsbA or DsbC protein and the nucleic acid encoding the heterologous polypeptide, under conditions whereby expression of the nucleic acid encoding the DsbA or DsbC protein is induced prior to induction of the expression of the nucleic acid encoding the heterologous polypeptide, and under conditions whereby either both the heterologous polypeptide and the DsbA or DsbC protein are secreted into the periplasm of the bacteria or the heterologous polypeptide is secreted into the medium in which the bacterial cells are cultured; and

(b) recovering the heterologous polypeptide from the periplasm or the culture medium.

18 Claims, 3 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 3

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMIC | Draw. D. |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|

☐ 5. Document ID: MX 2003004161 A1, WO 200240650 A2, AU 200221815 A, US

20030049729 A1, NO 200302143 A, BR 200115344 A, HU 200301619 A2, CZ 200301657 A3,
KR 2003059252 A, SK 200300579 A3, EP 1407030 A2, JP 2004520018 W, ZA 200303547 A

L1: Entry 5 of 6

File: DWPI

May 1, 2004

DERWENT-ACC-NO: 2002-519376

DERWENT-WEEK: 200482

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TITLE: Producing active, correctly folded recombinant tissue plasminogen activator,
Kringle 2 serine protease in prokaryotic cells by expressing the protein-encoding
DNA operably linked to DNA coding for signal peptide OmpA

INVENTOR: GOETZ, F; MANOSROI, A ; MANOSROI, J ; TAYAPIWATANA, C ; WERNER, R ;
TAYAPIWANATANA, C

PRIORITY-DATA: 2000GB-0027779 (November 14, 2000)

PATENT-FAMILY:

| PUB-NO | PUB-DATE | LANGUAGE | PAGES | MAIN-IPC |
|--------------------------|--------------------|----------|-------|------------|
| <u>MX 2003004161 A1</u> | May 1, 2004 | | 000 | C12N009/00 |
| <u>WO 200240650 A2</u> | May 23, 2002 | E | 080 | C12N009/00 |
| <u>AU 200221815 A</u> | May 27, 2002 | | 000 | C12N009/00 |
| <u>US 20030049729 A1</u> | March 13, 2003 | | 000 | C12P021/02 |
| <u>NO 200302143 A</u> | July 7, 2003 | | 000 | C12N015/70 |
| <u>BR 200115344 A</u> | August 26, 2003 | | 000 | C12N009/00 |
| <u>HU 200301619 A2</u> | September 29, 2003 | | 000 | C12N009/00 |
| <u>CZ 200301657 A3</u> | October 15, 2003 | | 000 | C12N009/00 |
| <u>KR 2003059252 A</u> | July 7, 2003 | | 000 | C12N009/00 |
| <u>SK 200300579 A3</u> | January 8, 2004 | | 000 | C12N015/62 |
| <u>EP 1407030 A2</u> | April 14, 2004 | E | 000 | C12N015/62 |
| <u>JP 2004520018 W</u> | July 8, 2004 | | 116 | C12N015/09 |
| <u>ZA 200303547 A</u> | June 30, 2004 | | 099 | C12N009/00 |

INT-CL (IPC): A61 P 9/00; A61 P 9/10; A61 P 11/00; C07 K 19/00; C12 N 1/21; C12 N 5/10; C12 N 9/00; C12 N 9/64; C12 N 9/72; C12 N 15/09; C12 N 15/12; C12 N 15/58; C12 N 15/62; C12 N 15/70; C12 N 15/72; C12 N 15/74; C12 P 19/34; C12 P 21/02

ABSTRACTED-PUB-NO: WO 200240650A

BASIC-ABSTRACT:

NOVELTY - Producing (M1) extracellularly secreted, active, correctly folded, recombinant tissue plasminogen activator (tPA) (I), Kringle 2 serine protease molecule (K2S) (II), or their variants (Ia,Ib) in prokaryotic cells (C1) by using a (C1) containing and expressing vector comprising DNA encoding (I,II,Ia or Ib) operably linked to DNA coding for signal peptide OmpA or its functional derivative, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a DNA molecule (III) coding for the OmpA protein or its functional derivative, operably linked to a DNA molecule coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tPA;

(2) a fusion protein (IV) of OmpA and K2S, comprising a fully defined sequence of 377 amino acids (S8) as given in the specification, or its fragment, functional variant, allelic variant, a subunit, a chemical derivative or a glycosylation variant;

(3) a K2S protein (V) comprising a fully defined sequence of SEGN (S9) or its variant, fragment, functional variant, allelic variant, subunit, chemical derivative, fusion protein or glycosylation variant;

(4) a vector (VI) containing (III);

(5) a vector pComb3HSS (VII) containing (III), where the expression of the gp III protein is suppressed or inhibited by deleting the DNA molecule encoding the gp III protein or by a stop codon between the gene coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tissue plasminogen activator protein and the gp III gene; and

(6) a prokaryotic host cell (VIII) comprising (III), (VI) or (VII).

ACTIVITY - Cerebroprotective; Cardiant; Thrombolytic.

No biological data is given.

MECHANISM OF ACTION - Mediator of fibrin formation and clot dissolution.

USE - M1 is useful for producing recombinant DNA-derived tissue plasminogen activator (tPA), Kringle 2 serine protease molecule (K2S), or variants of tPA or K2S molecule in a prokaryotic cell such as *Escherichia coli*. (III), (VI), (VII) or (VIII) are used in the method for producing a polypeptide with the activity of tPA protein. Preferably, the molecules are useful in (M1) (all claimed).

The DNA molecules, vectors or host cells are useful for producing a polypeptide having the activity of tissue plasminogen activator. Recombinant DNA-derived polypeptides from (M1) are useful for manufacturing a medicament for treating stroke, cardiac infarction, acute myocardial infarction, pulmonary embolism, any artery occlusion such as coronary artery occlusion, intracranial artery occlusion (e.g., arteries supplying the brain), peripherally occluded arteries, deep vein thrombosis, or related diseases associated with unwanted blood clotting.

ADVANTAGE - The use of the signal peptide OmpA alone and/or in combination with the N-terminal amino acids SEGN (S9)/SEGNSD (S10) translocate the recombinant DNA-derived tPA, tPA variant, K2S molecule or K2S variant to the outer surface and facilitates the release of the functional and active molecule into the culture medium to a greater extent than any other known method. Before crossing the outer membrane, the recombinant DNA-derived protein is correctly folded, the signal peptide is cleaved off to produce a mature molecule and the efficiency of signal peptide removal is very high and leads to correct folding of the recombinant DNA-derived protein.

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | RIMC | Draw. D |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|---------|
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|---------|

☐ 6. Document ID: MX 2003004162 A1, WO 200240696 A2, AU 200221824 A, US 20030013150 A1, EP 1343909 A2, KR 2003051821 A, JP 2004513648 W

L1: Entry 6 of 6

File: DWPI

May 1, 2004

DERWENT-ACC-NO: 2002-471625

DERWENT-WEEK: 200482
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TITLE: Producing recombinant DNA-derived kringle 2 plus serine protease, comprises using a prokaryotic cell expressing a vector having a DNA coding for a heterologous protein operably linked to a DNA coding for the signal peptide OmpA

INVENTOR: GOETZ, F; MANOSROI, A ; MANOSROI, J ; TAYAPIWATANA, C ; WERNER, R

PRIORITY-DATA: 2000GB-0027782 (November 14, 2000)

PATENT-FAMILY:

| PUB-NO | PUB-DATE | LANGUAGE | PAGES | MAIN-IPC |
|--------------------------|--------------------|----------|-------|------------|
| <u>MX 2003004162 A1</u> | May 1, 2004 | | 000 | C12P021/02 |
| <u>WO 200240696 A2</u> | May 23, 2002 | E | 052 | C12P021/02 |
| <u>AU 200221824 A</u> | May 27, 2002 | | 000 | C12P021/02 |
| <u>US 20030013150 A1</u> | January 16, 2003 | | 000 | C12P021/02 |
| <u>EP 1343909 A2</u> | September 17, 2003 | E | 000 | C12P021/02 |
| <u>KR 2003051821 A</u> | June 25, 2003 | | 000 | C12P021/02 |
| <u>JP 2004513648 W</u> | May 13, 2004 | | 079 | C12N015/09 |

INT-CL (IPC): C12 N 5/06; C12 N 15/09; C12 N 15/87; C12 P 19/34; C12 P 21/02

ABSTRACTED-PUB-NO: WO 200240696A

BASIC-ABSTRACT:

NOVELTY - Producing recombinant DNA-derived heterologous protein in prokaryotic cells, where the heterologous protein is secreted extracellularly as an active and correctly folded protein and the prokaryotic cell contains and expresses a vector comprising the DNA coding for the heterologous protein operably linked to the DNA coding for the signal peptide OmpA or its functional derivative, is new.

USE - The method is useful for commercial large-scale production of heterologous proteins, e.g. K2S, in prokaryotic cells, and is generally applicable in the expression of several different proteins and polypeptides which do not require mammalian glycosylation in prokaryotic host cells. The method may also be used to obtain DNA sequences of a protein of interest to be expressed from databases and cloned for use.

ADVANTAGE - Unlike previous methods, the new method is commercially applicable for large-scale production of heterologous proteins, and is more cost effective.

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|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. D. |
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Terms

ompa same (tissue plasminogen activator or tpa or t-pa or k2s or
kringle adj2 2 adj2 serine)

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☐ 1. Document ID: US 20040018586 A1

Using default format because multiple data bases are involved.

L6: Entry 1 of 3

File: PGPB

Jan 29, 2004

PGPUB-DOCUMENT-NUMBER: 20040018586

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018586 A1

TITLE: Method for refolding proteins containing free cysteine residues

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|------------|-------|---------|---------|
| Rosendahl, Mary S. | Broomfield | CO | US | |
| Cox, George N | Louisville | CO | US | |
| Doherty, Daniel H | Boulder | CO | US | |

US-CL-CURRENT: 435/68.1; 435/69.4

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Drawings |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
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☐ 2. Document ID: US 20030049729 A1

L6: Entry 2 of 3

File: PGPB

Mar 13, 2003

PGPUB-DOCUMENT-NUMBER: 20030049729

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049729 A1

TITLE: Methods for large scale production of recombinant DNA-Derived TPA or K2S molecules

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|------------|-------|---------|---------|
| Manosroi, Jiradej | Chiang Mai | | TH | |
| Manosroi, Aranya | Chiang Mai | | TH | |
| Tayapiwatana, Chatchai | BKK | | TH | |
| Goetz, Friedrich | Tuebingen | | DE | |

Werner, Rolf-Guenther

Biberach

DE

US-CL-CURRENT: [435/69.1](#); [435/252.33](#), [435/320.1](#), [435/488](#), [435/91.2](#)

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|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D |
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☐ 3. Document ID: US 20030013150 A1

L6: Entry 3 of 3

File: PGPB

Jan 16, 2003

PGPUB-DOCUMENT-NUMBER: 20030013150

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030013150 A1

TITLE: Methods for large scale protein production in prokaryotes

PUBLICATION-DATE: January 16, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|------------|-------|---------|---------|
| Manosroi, Jiradej | Chiang Mai | | TH | |
| Manosroi, Aranya | Chiang Mai | | TH | |
| Tayapiwatana, Chatchai | Bkk | | TH | |
| Goetz, Friedrich | Tuebingen | | DE | |
| Werner, Rolf-Guenther | Biberach | | DE | |

US-CL-CURRENT: [435/69.1](#); [435/320.1](#), [435/325](#), [435/455](#), [435/91.2](#)

| | | | | | | | | | | | | |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D |
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ompa same (tissue plasminogen activator or tpa or t-pa or k2s or
kringle adj2 2 adj2 serine)

3

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